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13. ABSTRACT *(Maximum 200 words)*

This research investigates the effect of the hormone melatonin on the circadian clock of mammals, by examining daily activity/rest cycles and body temperature rhythms in melatonin-infused Siberian hamsters, under a variety of environmental lighting conditions. In experiments simulating jet-lag conditions, melatonin significantly accelerated re-adjustment of sleep/wake rhythms to phase-shifted light cycles. Within days after an 8-hr phase-advance of the light/dark cycle, all melatonin-treated hamsters, but none of the saline-treated controls, had achieved the proper phase relationship with the new photoschedule. These results are consistent with reports of melatonin treatment reducing jet lag in humans. Under conditions of constant darkness, daily melatonin infusions synchronized the hamster activity/rest rhythm. In constant light, melatonin also acted as a weak entraining agent and prevented the internal desynchronization which occurs in Siberian hamsters and in many mammals exposed to constant light. These results offer encouragement about Siberian hamsters as an appropriate model system to investigate melatonin action on the circadian clock. Further studies should increase our understanding of the role of melatonin receptors localized within the clock of rodents and humans, and should supply needed information relevant to the expanding clinical use of melatonin to treat a variety of temporal disorders, such as jet-lag.

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MELATONIN ACTION ON THE CIRCADIAN PACEMAKER IN SIBERIAN HAMSTERS

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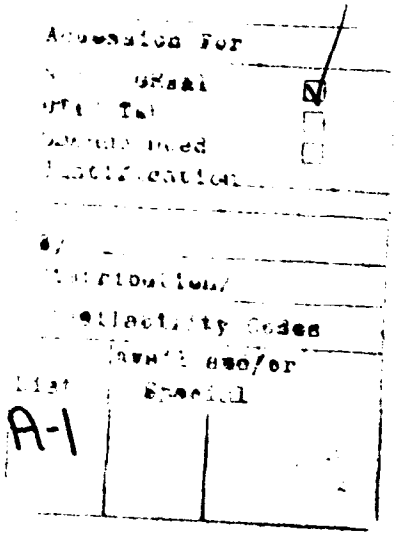

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A) OBJECTIVES OF RESEARCH EFFORT:

The overall goal of this project is to characterize the interaction of melatonin with the circadian clock of mammals, using the melatonin-infused Siberian hamster as a model system. The significance of this project is to provide basic information relevant to the recently expanding clinical use of melatonin to treat temporal disorders (including jet-lag) as well as to ellucidate the function of specific melatonin receptors localized within the circadian clock (the suprachiasmatic nuclei, or SCN) of rodents and humans. The project has employed the following specific approaches:

- To determine if melatonin is an entraining agent ("Zeitgeber") for locomotor activity and body temperature rhythms in Siberian hamsters. This was to be ascertained under conditions of constant light (Part 1) and constant darkness (Part 2, of the original grant proposal).
- To determine if melatonin treatment protects the multioscillatory circadian system from "internal desynchronization" which occurs in numerous species after prolonged exposure to constant light (Part 1). Internal desynchronization is characterized by divergence of two or more rhythms (such as the wheel-running vs. temperature rhythms; or such as the "splitting" of morning vs. evening components of the wheel-running rhythm). If melatonin treatment affects the divergence of such rhythms, this suggests the hormone may affect strength of coupling among component oscillators of the circadian pacemaker system..
- To determine if melatonin treatment affects the rate of adjustment to phase-shifted light cycles (Part 3: the "jet lag experiments"). If so, then continued use of the Siberian hamster model system should be fruitful for characterizing melatonin treatment of jet lag and other temporal disorders in humans.
- To determine if there is a daily rhythm of receptivity to melatonin in the SCN by direct measurement of high affinity melatonin receptors at different times of day in the anterior hypothalamus and other brain sites of melatonin action (Part 4).

B) STATUS OF RESEARCH EFFORT:

For each section, (Part 1 through 4, as listed in the grant proposal) progress and results are itemized below. Figures and references cited are to be found at end of text.

Part 1. Melatonin infusions under conditions of constant light

Several studies have been performed to reassess the role of melatonin as an entraining agent in constant light. In two trials of Experiment 1a, pinealectomized juvenile male hamsters received 10 hour subcutaneous infusions of melatonin (100 ng/day) or saline every 24.6 hours, and wheel-running rhythms were monitored under conditions of dim constant white light (<10 lux). As in earlier studies, the incidence of splitting of the wheel-running activity was less for melatonin- infused hamsters than in the saline group, suggesting that melatonin may prevent LL-induced internal desynchronization. In one, but not both of these studies, mean tau values for melatonin vs saline treated hamsters were significantly different, indicating a subtle entraining effect of melatonin on the wheel-running activity rhythm. Further investigation of this question is now ongoing, using the Dataquest system for monitoring body temperature rhythms as well as locomotor activity. Two pilot experiments have been run to test the system prior to hormone infusion studies, using 60-day old Siberian hamsters implanted with Minimitter (XM-FH) telemeters. (1) After 45 days exposure to dim LL (7.5 - 15 lux), the incidence of splitting was lower than previously observed with juvenile male hamsters (< 20% vs. >90%), suggesting an age effect on threshold light intensity required for splitting. When

splitting occurred, it was seen in both activity and temperature rhythms (Figure 1). Whereas wheel-running activity was often diminished in constant light, the body temperature rhythm remained robust. Thus, the temperature measurements will now permit use of higher light intensities than previously possible, to potentially increase the incidence and magnitude of internal desynchronization. Plans are made to test the effect of melatonin manipulations under these conditions in adult males. (2) After a 6-hr phase advance of the light/dark cycle, a transient dissociation of the temperature and wheel-running rhythms was observed, suggesting that the two rhythms may be governed by separate oscillators, and may be differentially affected by melatonin treatment.

Tentative Conclusions:

Data from these and earlier studies suggest that melatonin may be acting as a weak Zeitgeber; capable of entraining the wheel-running rhythm but only when "T" is close to tau. Under these conditions, melatonin prevented LL-induced splitting of the activity rhythm, suggesting an effect of the hormone on strength of coupling between multiple oscillators. The unusually rapid splitting of locomotor activity observed in juvenile Siberian hamsters exposed to dim LL deems these animals as good model system for studying internal desynchronization. With the ability to measure temperature and activity rhythms, it should be possible to further investigate melatonin effects on circadian organization.

Part 2. Melatonin infusions under conditions of constant darkness

Several studies have been completed in which 10 hr melatonin (or saline) infusions were administered every 24 hrs to pinealectomized juvenile male hamsters maintained in constant dim red light (<1 lux). Fig. 2 shows the experimental design for one such study and locomotor activity records for representative saline- and melatonin-infused individuals. Saline-treated hamsters free-ran through the infusion interval with a periodicity less than 24 hrs (mean tau value of 23.56 $\pm$ .05 hrs). In contrast, after the first ten days of infusion, activity onset of melatonin-infused hamsters appeared to stabilize several hours prior to the onset of the daily melatonin pulse. The mean tau value for the melatonin group (23.91 $\pm$ .04 hrs) approached that of the infusion periodicity, and was significantly greater ($p<.01$) than that of the saline group (Fig. 3). These and similar results from other studies indicate a subtle entraining effect of melatonin.

In a modification of Experiment 2a, the duration of the melatonin infusion was gradually increased from 6 to 10 hours (in order to more closely simulate a natural change in daylength than with the above abrupt transition into long-duration melatonin pulses). No major difference in the rate of entrainment to melatonin was observed, nor in the phase angle of activity onset relative to the melatonin pulse, when compared to results of Expt. 2a .

Conclusions: The entraining effect of melatonin infusions observed in DD is consistent with a role of the hormone as a weak Zeitgeber, and is in agreement with results of daily melatonin injections in pineal-intact rats (Redman et al., 1983; Cassone et al., 1986)

Yet to be determined is whether there is a phase response curve to melatonin, ascertainable by experiments using short melatonin pulses in pineal-intact and pinealectomized hamsters (e.g. Expts. 2c, 2d), and whether melatonin infusions might affect the strength of coupling between morning and evening oscillators, as in Expt. 2e.

Part 3. Melatonin infusions during phase-shifting of the light cycle : *The Jet-Lag Experiments*

Several studies have been completed, of the type described in section 3b of the proposal, and the results are very encouraging. In Experiment 3b #1, juvenile hamsters were subjected to a 6-hr phase advance of the LD16:8 light cycle. Two days before the phase shift, the hamsters were subcutaneously cannulated, and 6-hr daily infusions of melatonin (at 60 ng per day) or saline were begun at the phase indicated in Fig. 4 . The infusions were continued for 7 days after the phase-shift, at which time the infusion pumps were turned off and the hamsters were released into constant darkness for assessment of the phase of activity onset. Wheel running records of two individual hamsters are presented in Fig. 5. All the melatonin treated animals (n=10) phase-advanced in response to the phase shift, and within a few days had acquired the expected phase relationship with the new light cycle. In contrast, only 4 of the 8 saline animals phase-advanced. The other 4 delayed (as did the individual in Fig. 5) and showed a mean activity onset value (extrapolated from the DD free run) significantly different (05.001 + 01.609 hrs) from mean onset for the melatonin group (12.903 + 0.219 hrs; p=.0188).

In a subsequent study, several changes in experimental paradigm were tested. A more challenging 8-hr phase advance was employed, to increase the likelihood of a homogenous "jet-lag" response in the saline group. The melatonin dosage was increased to 600 ng/day, to be ten-fold higher, rather than just at the threshold dose known to cause entrainment in pineal-intact rats (Cassone et al, 1986). In addition, the timing of the 6 hr melatonin infusions was modified to simulate the phase of melatonin treatments reported to diminish jet lag in a group of scientists flying from San Francisco to London (Arendt et al., 1987). In Fig. 6, this experimental paradigm is schematically represented. Results of this study were striking, as shown by representive individual activity records (Fig. 7) and by mean activity onset data (Fig. 8). All the melatonin animals (n=9) phase advanced rapidly, and within 4 days after the phase shift had achieved the expected phase relationship with the new light cycle. In contrast, all saline-treated hamsters (n=8) phase delayed, and were showing atypical diurnal activity before release into constant darkness. Mean activity onset for this group occurred several hours after dawn in the new light cycle (03.347 + 1.944 hrs), significantly different (p=.0017) than for the melatonin group (11.061 + .925 hrs).

Conclusions:

Melatonin significantly accelerated re-entrainment of the circadian system to a phase-shifted light cycle. These results are consistent with the effectiveness of melatonin to ameliorate symptoms of jet-lag in humans. Further studies of this system should increase our understanding of the role of melatonin receptors in the mammalian SCN and should supply needed information relevant to the expanding clinical use of melatonin to treat temporal disorders.

Part 4: Daily rhythm of receptivity to melatonin in the SCN

We have now collected approximately 800 hamster brains (flash-frozen in 2-methyl butane, stored at - 80 C). The experimental groups listed below will be used to test for a rhythm of melatonin receptors in the presence or absence of endogenous pineal melatonin. In addition, an approximately equal number of brains has for been collected at various times of the light/dark cycle for validating receptor assay procedures. The receptor measurements are being performed in collaboration with Dr. Vincent Cassone at Texas A&M University.

pinx females	sampled every 2 hours for 24 hours on LD 16:8 light cycle	n =120
sham pinx females	" " " " " " "	n =120
intact males	" " " " " " "	n =120
intact males sampled at night with lights remaining on		n = 40

C. MANUSCRIPTS IN PREPARATION

Darrow J.M., Doyle S.E. and Goldman, B.D. *Effects of daily melatonin infusions on the circadian clock and reproductive system of Siberian hamsters exposed to constant light.*

Darrow, J.M. and Doyle, S.E. Effects of daily melatonin infusions on locomotor activity rhythms of Siberian hamsters exposed to constant darkness

Darrow, J.M. and Doyle, S.E. Melatonin infusions accelerate entrainment of Siberian hamsters to phase-shifted light cycles.

D. PROFESSIONAL PERSONNEL

Susan E. Doyle, Wellesley College Honors graduate

E. INTERACTIONS

DATA PRESENTATION:

Darrow, J.M. and S. E. Doyle, Melatonin infusions in wheel-running Siberian hamsters in constant darkness: Possible entrainment of the locomotor activity rhythm. Annual Meeting Soc. Research Biological Rhythms, Jacksonville, Fla, 1990.

Darrow, J.M. Invited seminar speaker at Northeastern University, Vassar College, Wellesley College, Williams College, 1990-91.

COLLABORATIONS:

With Dr. Vincent Cassone, Texas A &M University
With Dr. Fred C. Davis, Northeastern University
With Dr. Eve S. Hiatt, Northeastern University

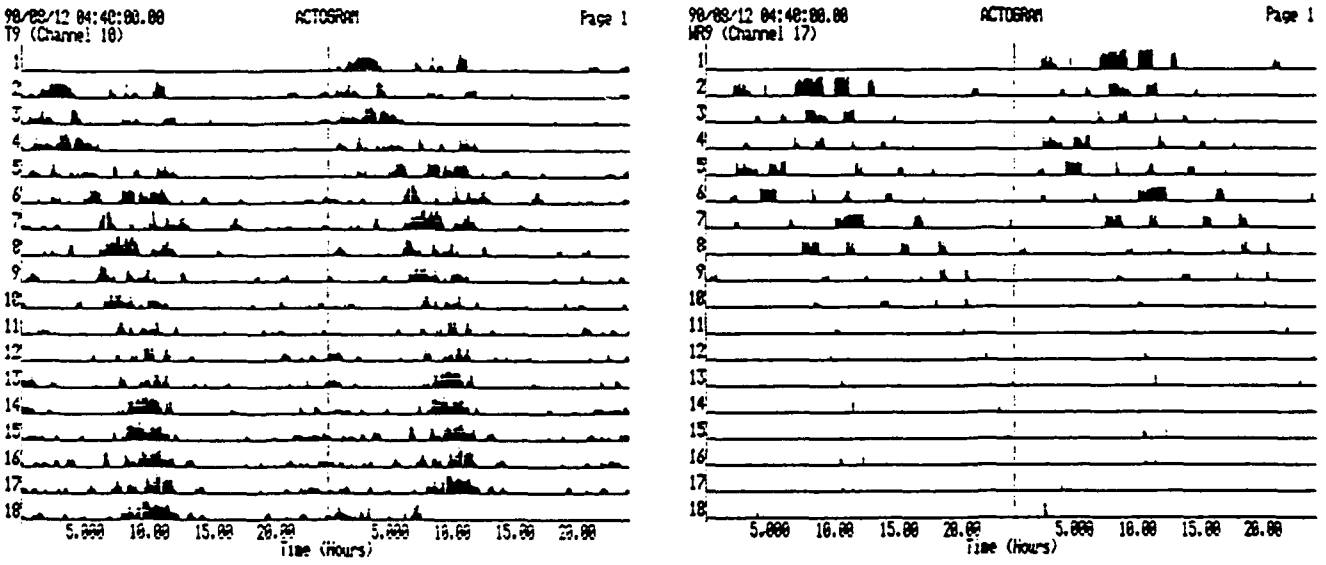


FIGURE 1 Double-plotted records of body temperature (left panel) and wheel-running activity (right panel) for an adult male Siberian hamster free-running in constant dim white light (<15 lux). Splitting of both rhythms was evident by day 10 (28 days after exposure to LL). Whereas wheel-running activity is often diminished in constant light, the body temperature rhythm (monitored by the Dataquest Minimitter system) was robust and splitting could be clearly detected.

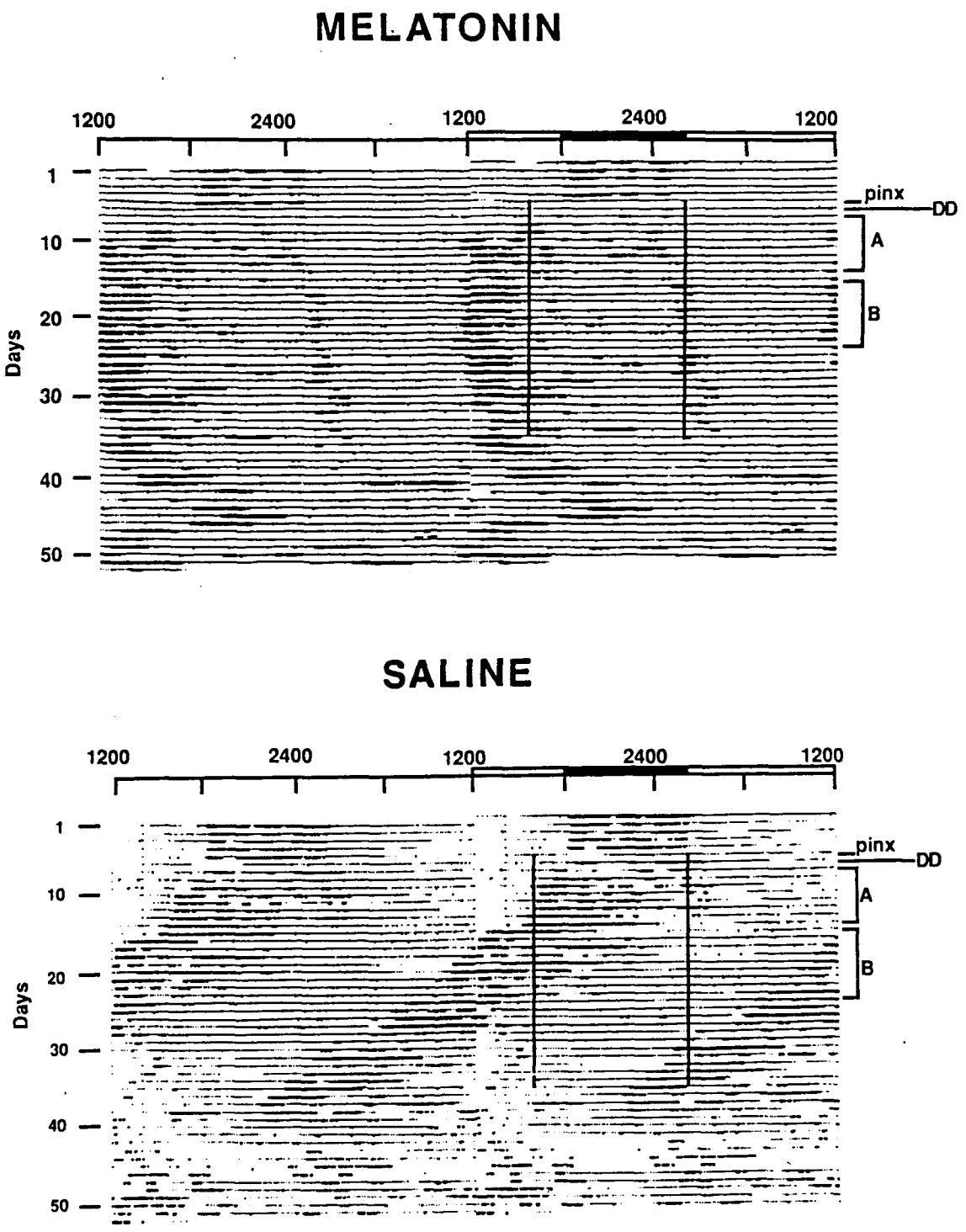


FIGURE 2 Wheel-running activity records for saline and melatonin infused hamsters in constant darkness (DD). Juvenile male hamsters were pinealectomized and cannulated on day 6 of the record, then released from LD16:8 into constant darkness on day 7. Daily infusions began on the afternoon of surgery, for a 10-hour duration as indicated by the parallel vertical lines (melatonin dose = 10 ng/hour). After 4 weeks, infusion pumps were switched off and activity rhythms monitored for another 15 days before autopsy. Brackets at the side of each record indicate intervals from which activity onset times were measured (Interval A: from 3-10 days; Interval B: from 11-21 days after daily infusions began).

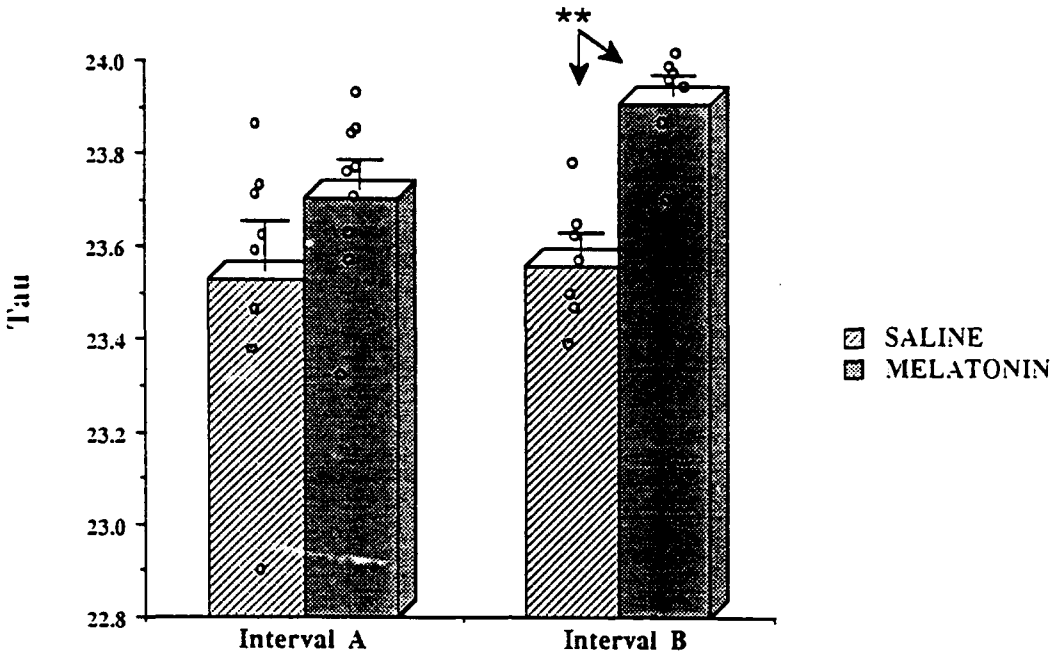


FIGURE 3 Mean tau values for saline and melatonin treated hamsters measured at Intervals A and B (see Fig. 2) during infusions in constant darkness. Open circles denote tau values of individual hamsters, horizontal lines above the bars indicate S.E.M.

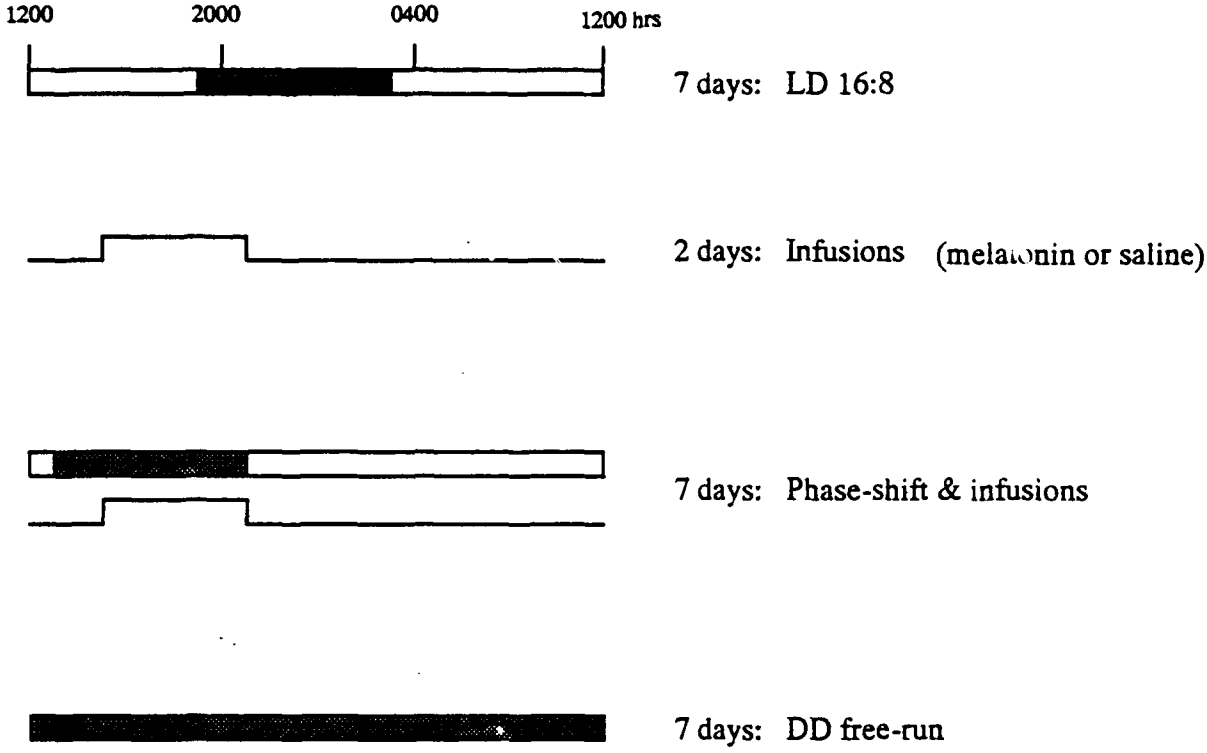
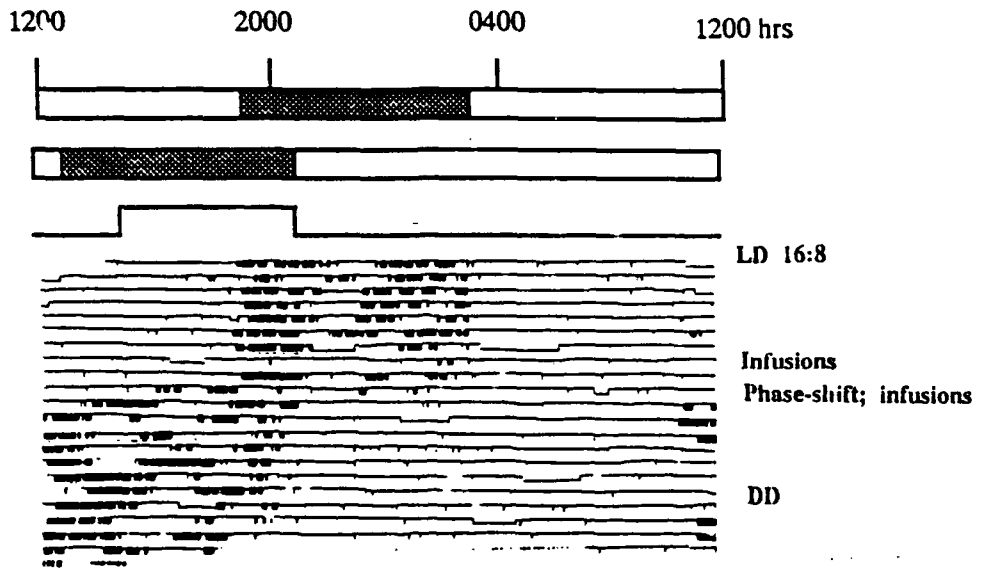


FIGURE 4 Experimental protocol for melatonin treatment during a 6-hr phase advance of the light cycle. After juvenile male Siberian hamsters were acclimated to running-wheel cages in LD16:8 for 7 days, they were subcutaneously cannulated and infused with melatonin (60 ng/day) or saline for 6 hrs/day (beginning at 1400 hrs), as indicated by the square wave. Two days later, the light cycle was phase-advanced by 6 hrs, and the daily infusions continued for 7 days. After this time, the infusion pumps were turned off and the animals allowed to free-run in constant darkness for 7 days.

 # 9 Melatonin



#12 Saline

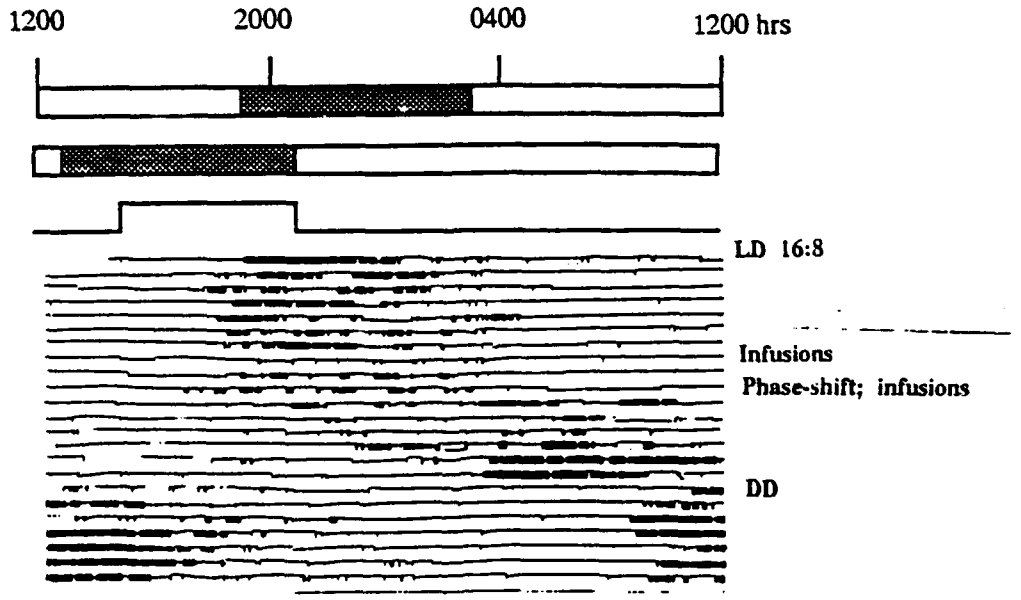


FIGURE 5 Wheel-running activity records for saline and melatonin-infused hamsters during a 6-hr phase advance of the light cycle. Experimental design is described in Figure 4.

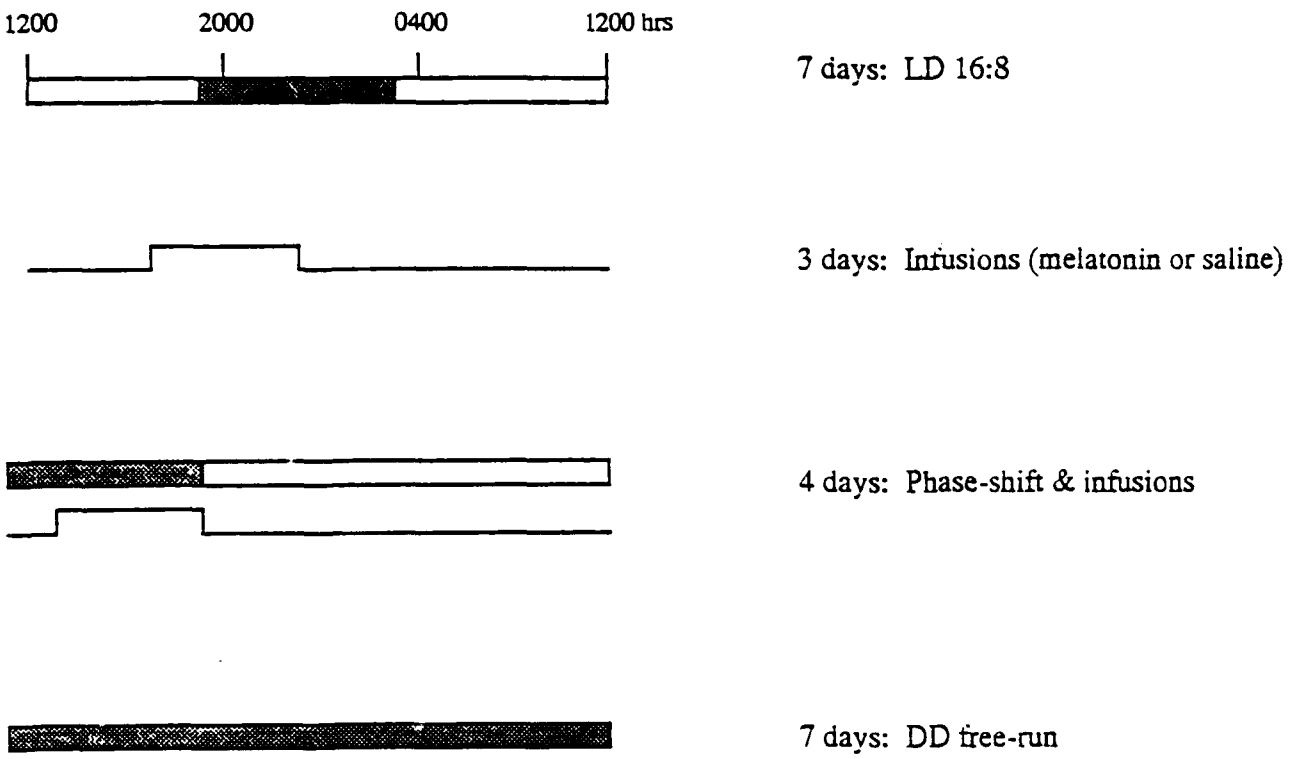


FIGURE 6 Experimental protocol for melatonin infusions during an 8-hr phase advance of the light cycle. After juvenile male Siberian hamsters were acclimated to the running-wheel cages in LD 16:8 for 7 days, they were subcutaneously cannulated and infused with melatonin (600 ng/day) or saline for 6 hours/day, beginning at 1700 hrs) as indicated by the square wave. Three days later, the light cycle was phase-advanced by 8 hours, and daily infusions (beginning at 1400 hrs) continued for an additional 4 days, as indicated by the second square wave. After this time, infusion pumps were turned off and hamsters were released into constant darkness for determination of activity onset in the absence of a light : dark cycle.

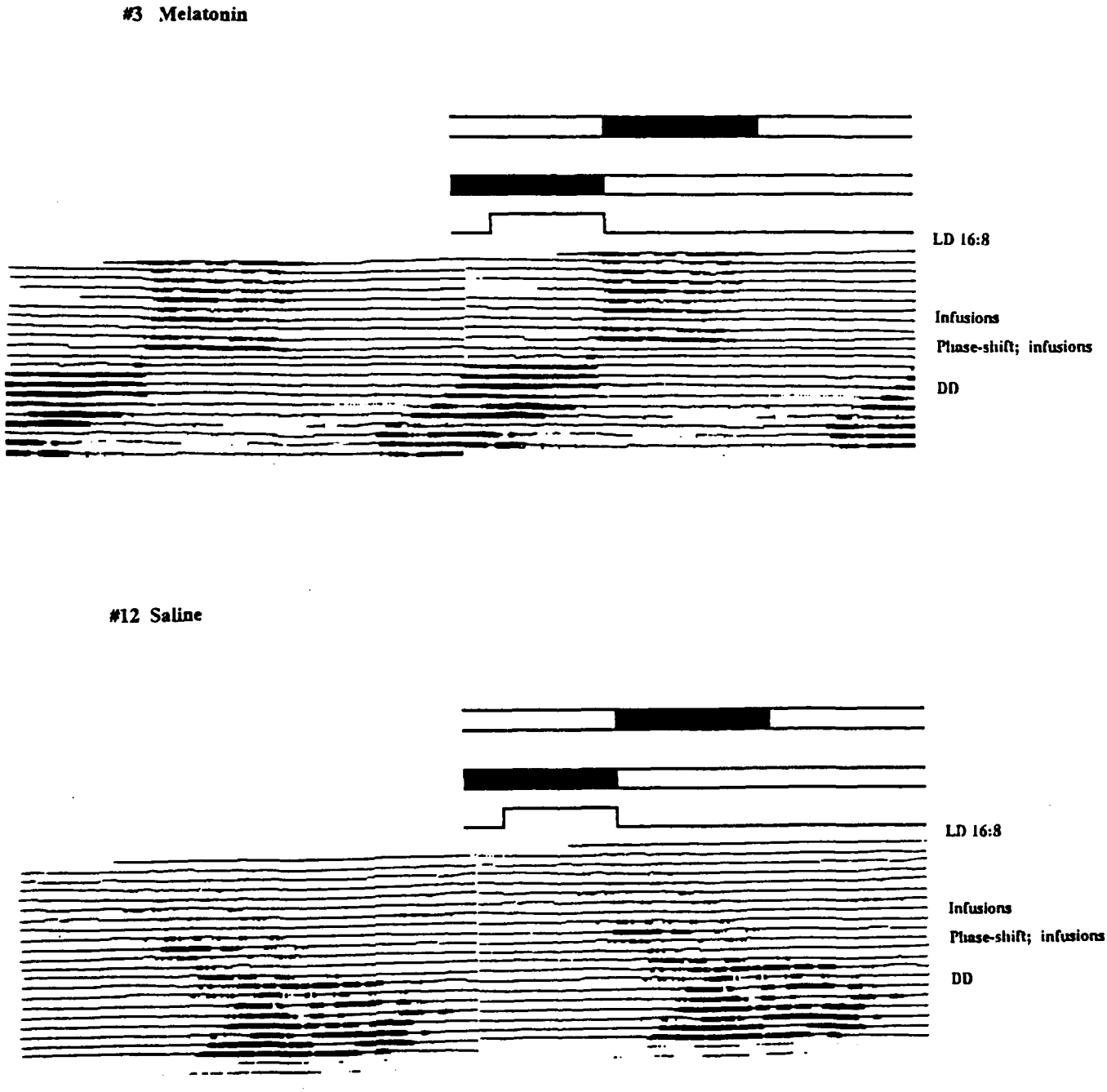


FIGURE 7 Wheel-running activity records for saline and melatonin-infused hamsters during an 8-hr phase advance of the light cycle. Experimental design is described in Figure 6.

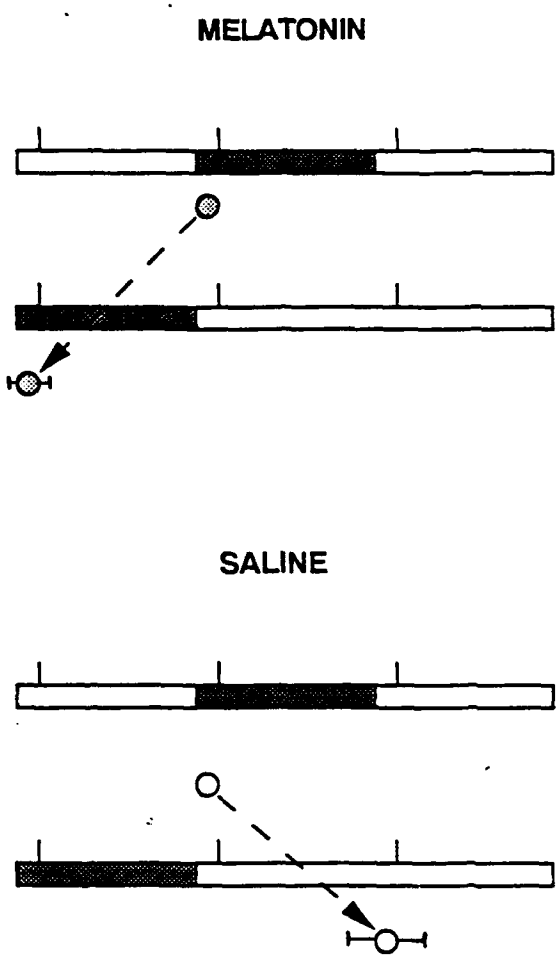


FIGURE 8 Mean activity onset relative to the light:dark cycle (LD16:8) before and after the 8-hour phase-shift. All melatonin-infused animals (n=9) phase advanced and rapidly acquired the expected phase relationship to the new photoschedule; all saline-treated hamsters (n=8) phase delayed, and showed an atypical diurnal activity pattern.